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## Pathogen metabolite checkpoint: NHR on guard

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### Summary blurb

*How can beneficial microorganisms be distinguished from pathogenic ones? In their study, Peterson et al. discovered that a specific phenazine, which is part of a family of toxic metabolites expressed by pathogenic bacteria, is detected by *Caenorhabditis elegans* by directly binding to a nuclear hormone receptor, promoting the expression of detoxifying enzymes and immunity-related genes, thus protecting the worm (1).*

Due to co-evolution between organisms, the line between beneficial versus pathogenic microbes can sometimes be hard to draw, and can depend on the context. Being able to distinguish a microbe with a positive impact on the host from a pathogenic microbe, is, however, crucial for survival. *C. elegans* lacks classic pattern-recognition receptors, but Peterson et al. discovered a cue that these nematodes use to identify harmful bacteria. Certain pathogenic bacteria express a specific metabolite of the phenazine family. This can be directly sensed by a nuclear receptor in the gut epithelium of *C. elegans*, allowing fast transcription of protective genes, including detoxifying enzymes and immune-related genes (1).

How to distinguish beneficial versus harmful interactions with microbes? There are a variety of ways that organisms use to know when they are being infected. One relies not on direct detection of a pathogen, but rather on the consequences of infection. There is a striking example from *Drosophila*, when a fungal protease activates the Toll signalling pathway, by cleaving the endogenous pre-ligand, Spaetzle (2). Such detection systems are varied and widespread. In *C. elegans*, for example, disruption of the apical extracellular matrix, or perturbation of ribosomal, mitochondrial or nucleolar function leads to the expression of defence genes, through mechanisms that are more or less understood (3, 4). In parallel, organisms have different pattern recognition receptors (PRR) that directly recognize non-self. In mammals, for example, this can be through Toll-like receptors (TLR). These bind a range of archetypical microbial moieties, including LPS from Gram-negative bacteria. Peptidoglycan, another bacteria cell wall component, is detected by PGRP receptors in insects and NOD-like receptors and TLR2 in mammals. Curiously, with the exception of the detection of viral double-stranded RNA, despite intense research, examples of direct pathogen recognition have been lacking in *C. elegans* (3). The paper by Peterson *et al.* in this issue of Immunity, fills in part this lacuna as it describes a novel PRR in *C. elegans*, a nuclear hormone receptor (NHR) that detects a specific toxic bacterial metabolite.

As well as the examples of indirect “surveillance” immunity cited above, *C. elegans* is able to detect a range of bacterial metabolites that can impact its behaviour and physiology (5). In the context of *Pseudomonas aeruginosa*, arguably the best studied *C. elegans* pathogen, it is able to avoid the virulent strain PA14 through the sensing, by a pair of chemosensory neurons, of phenazines, a group of bacterial toxic metabolites (5). Phenazine toxicity is due to an ability to interrupt the electron transport chain in the mitochondria. It also maintains adequate redox conditions in bacterial biofilms, thus sustaining bacterial survival. Peterson *et al.* now demonstrate through an elegant combination of

genetics, in both the host and the pathogen, that detection of the phenazine-1-carboxamide (PCN) elicits a protective immune response (1). As metabolites can be sensed by NHR, the authors screened by RNAi almost all of the 274 genes encoding NHRs in the worm genome. They found NHR-86/HNF4 to be required cell-autonomously in the gut for immune response induction by PCN. The authors demonstrated by chromatin immunoprecipitation that activated NHR-86 binds directly to the promoters of infection-response genes, independent of intermediary signalling pathways. Very strikingly, combining biophysics and genetics, the authors demonstrated that PCN binds directly to NHR-86. They identified a specific binding pocket that is also used by a synthetic immunostimulatory molecule R24 that triggers the same transcriptional responses as PCN (1).

The toxin thus has 2 antagonist outcomes in the host: killing it and protecting it by activating immune response through PRR recognition. Indeed, Peterson et al show that PCN is only mildly toxic in wild-type worms, but is highly detrimental in NHR-86 depleted worms, probably because the latter worms lack a detoxification response. In pathogenic bacteria, PCN levels correlates with levels of other toxic phenazine metabolites, such as phenazine-1-carboxylic acid, pyocyanin, and 1-hydroxyphenazine. The fact that PCN is mildly toxic in itself in WT worms suggests that it might be used by the worm as a cue to assess the potential harm of the bacterial strain, which depends on the synthesis of several phenazine metabolites. Moreover, supplementation of PCN in wild-type, but not NHR-86-depleted worms, prolongs survival of *C. elegans* after bacterial infection, presumably a reflection of a form of immune priming (1).

One limitation of the study is that, without PCN supplementation, the lack of NHR-86 did not affect worms' survival following bacterial infection. The authors suggest that the levels of PCN produced by the bacteria are too low in the growth conditions used, and do not reach the concentration required to bind and activate NHR-86, and thus the NHR-86-mediated immune and detoxifying effect. Their study shows, however, that PCN is produced specifically by virulent *P. aeruginosa*, providing a mechanism by which harmless and harmful bacteria can be distinguished.

Could the recognition of toxic bacterial metabolites through direct binding on NHR be a conserved mechanism to direct host defences towards potential pathogens? Phenazines are part of a bacterial quorum sensing system, and are secreted in a context of high, and thus potentially harmful, microbial density. Importantly, phenazines are required for full virulence of *P. aeruginosa* in mice by promoting cell toxicity and neutrophil recruitment (6). In mice, phenazines are recognized by the host' aryl hydrocarbon receptor (AhR), another ligand-activated transcription factor belonging to the bHLH superfamily, to regulate the transcription of immune-related genes and detoxifying enzymes (7). AhR-deficient mice are thus more susceptible to bacterial infection with *P. aeruginosa* (6). Other bacterial metabolites can be sensed as danger signals in the context of pathogenic infections. For instance, the major histocompatibility complex class I-related (MR1) receptor binds to metabolites of the Vitamin B2 biosynthesis pathway, which is conserved in bacteria and yeast. MR1 receptor on the presenting/infected cell further activates the T-cell receptor on MAIT T cells, an 'innate' like population of T cells to, allowing the production of cytokines (e.g. IFN $\gamma$ , TNF $\alpha$ , IL17) and pathogen clearance (8).

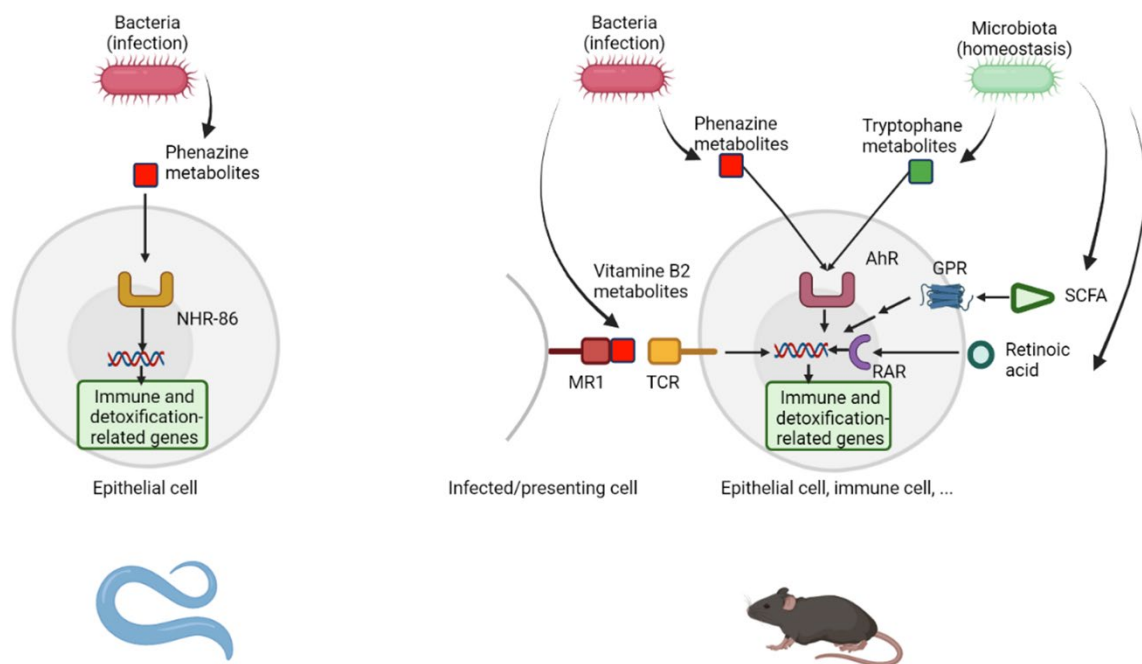
Depending on the context, microbial metabolites can, however, also be beneficial, by educating the immune system, protecting host barriers, and setting a regulatory tone as exemplified by the positive effect of the microbiota on host responses in mammals. For instance, AhR also recognizes ligands derived from the microbiota, like tryptophan and arginine derivatives, which are crucial for immune regulatory functions, including in the brain (microglia-astrocyte interplay) and in the gut (tolerogenic DCs production of IL10) (9). Immune regulation can be modulated by other types of metabolites including short-chain fatty acids (SCFA), that are the most abundant microbial metabolites and are derived from the fermentation of non-digestible polysaccharides by the microbiota. SCFA can act through G-protein coupled receptors (like Gpr43, 41, 409a) or through epigenetic modifications (inhibition of histone deacetylases). SCFA enhances myeloid output and

promotes macrophage antimicrobial functions. They also promote intestinal Tregs, IgA+ B cells and ILC3s, as well as memory CD8+ T cells, thus promoting host protection (9, 10). Vitamin metabolites, such as retinoic acid, also have tolerogenic properties by promoting Tregs and IgA production by B cells. Similarly, other bacterial metabolites (ATP, NAD, lactate, pyruvate, folate, histamine, dopamine, polyamines and bile acid derivatives) have been shown to have regulatory and anti-inflammatory properties in steady-state conditions (9, 10).

Thus, microbial metabolites trigger immune functions that are at play at steady state to keep the microbiota in check, but can also promote pathogen clearance in case of microbial invasion. The location, nature, concentration and effects of bacterial products are all involved in the distinction between beneficial and pathogenic bacteria. And it is now recognized that microbial metabolites are key molecules driving immune responses, in addition to components of the wall or genomic material. Beyond this, microbial metabolites are also involved in shaping organisms' physiology and behaviour, thus allowing an integrated and adapted response of the organism to its environment. Identifying an NHR as a direct guard of toxic metabolites extends the palette of PRR. NHRs have apparently expanded in *C. elegans* as it harbours 274 different genes, compared to only forty-eight in the human genome. What metabolites are recognised by these different NHRs, and whether any ligand-receptor pairs have been conserved across 600 million years will be an important area of research in the future.

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**Figure: Microbial metabolites sensing in health and disease.** In *C. elegans* (left), phenazine metabolites produced by pathogenic bacteria are recognized by the nuclear receptor NHR-86, leading to the expression of immune and detoxification-related genes, and worm protection from the infection. In mice (right), metabolites from pathogenic bacteria are also recognized. For instance, phenazine metabolites are recognized by aryl hydrocarbon receptor (AhR), and Vitamin B2 metabolites are presented by Major histocompatibility complex class I-related gene protein (MR1) and recognized by the T-cell receptor (TCR) of Mucosal-associated invariant (MAIT) T cells. Depending on the context, this can lead to modulation of host immune response as well as detoxification. In mice, metabolites from the microbiota are also sensed in homeostatic conditions by immune and non-immune cells, such as tryptophan metabolites by AhR, short-chain fatty acid by G-protein coupled receptor (GPR) and retinoic acid by the retinoic acid receptor (RAR), leading to immune education and regulatory pathways (created in BioRender.com).